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Studies on Dextranase. VI. Some Physicochemical Properties and Amino Acid Compositions of Dextranases from *Brevibacterium fuscum* var. *dextranlyticum* and *Penicillium funiculosum* IAM 7013[1a,b]

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Some physicochemical properties and amino acid compositions of dextranases from *Brevibacterium fuscum* and *Penicillium funiculosum* IAM 7013 were investigated. The values of 4.35S and 4.37S, respectively, were obtained for the sedimentation constant ($S_{20,w}$) of dextranases from *B. fuscum* and *P. funiculosum*, and their isoelectric points (pI) were also determined 4.17 and 4.19, respectively. Gel filtration on Bio Gel P-100 indicated the molecular weight 5.5×10^4 for *B. fuscum* and 4.4×10^4 for *P. funiculosum*, and their intrinsic viscosities were 0.038 dl/g and 0.027 dl/g, respectively.

Amino acid analysis suggested that dextranase of *B. fuscum* was found to be composed of more than 429 amino acid residues of 17 amino acids and the enzyme of *P. funiculosum* contained more than 349 of the residues of 18 amino acids. The composition of the enzymes was very similar except for two residues of cystine which were contained in only *P. funiculosum* dextranase.

Dextranase of *B. fuscum* contained 11 moles of neutral sugars as glucose and 3 moles of amino sugars as glucosamine per one mole of the enzyme, and in *P. funiculosum*, the former was 10 moles and the later was found to be 1 mole. The results of staining of the enzymes in disc electrophoresis gels by periodic acid and fuchsin-sulfite, and the observations above mentioned indicated that both dextranases were glycoprotein.

In the previous papers, we have reported that the purification and some enzymatic properties of dextranases from *P. funiculosum* IAM 7013[3-6] and *B. fuscum*.[7] Both enzymes were highly purified by ammonium sulfate fractionation, gel filtration on Bio Gel, ion-exchange cellulose chromatography and isoelectric focusing.[3,7] However, physicochemical properties and amino acid compositions of these dextranases have not been clarified.

In this paper, some physicochemical properties and amino acid compositions of the highly purified dextranases are described.

Materials and Methods

Enzyme——Dextranases of *B. fuscum* var. *dextranlyticum* and *P. funiculosum* IAM 7013 were purified from the culture filtrate according to the methods described in previous papers.[3,7] The preparation obtained were homogeneous in a disc electrophoresis. In the case of *P. funiculosum*, two active fractions were obtained from the culture filtrate and they were disignated *P. funiculosum* dextranases I (pI 3.98) and II (pI 4.19), respectively. However, their enzymatic properties were quite similar each other.[3] In this series of experiments, the later enzyme was used.

Determination of Sedimentation Constant——The samples were prepared as follows; *B. fuscum* and *P. funiculosum* dextranases were dissolved in 100 mM phosphate buffer (pH 7.5) and (pH 6.0) containing

1) *a*) Part V: M. Sugiura, A. Ito, T. Ogiso and K. Kato, *Chem. Pharm. Bull.* (Tokyo), **22**, 2953 (1974); *b*) This forms part 1C of "Studies on Enzymes" by M. Sugiura.
2) Location: *Ueno-sakuragi, 1-Chome, Taito-ku, Tokyo, 110, Japan.*
3) M. Sugiura, A. Ito, T. Ogiso, K. Kato and H. Asano, *Biochim. Biophys. Acta*, **309**, 357 (1973).
4) M. Sugiura and A. Ito, *Chem. Pharm. Bull.* (Tokyo), **22**, 1593 (1974).
5) M. Sugiura and A. Ito, *Chem. Pharm. Bull.* (Tokyo), **22**, 2941 (1974).
6) M. Sugiura, A. Ito, T. Ogiso and K. Kato, *Chem. Pharm. Bull.* (Tokyo), **22**, 2953 (1974).
7) M. Sugiura, A. Ito and T. Yamaguchi, *Biochim. Biophys. Acta*, **350**, 61 (1974).

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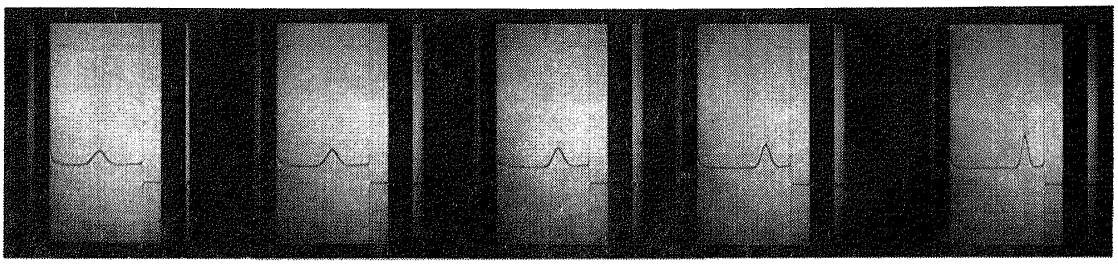
10 mM NaCl, respectively, and dialyzed against the same buffers at 4° for 24 hr. Ultracentrifugal analysis was carried out using a Hitachi Analytical Ultracentrifuge (model UCA-1), and the sedimentation constant was calculated by the method of Schachman.[8]

Isoelectric focusing, Molecular Weight and Viscosity Measurements——Estimation of isoelectric point was carried out by the method of Vesterberg and Svensson[9] using a carrier ampholyte (pH 3—5) at 1% concentration. *P. funiculosum* dextranase was fractionated into two preparations, dextranase I and II, by the procedure. Determination of molecular weight was carried out according to the procedure of Whitaker[10] using Bio Gel P-100. Viscosity was also measured in a Ostwald's viscometer at 25°.

Amino Acid Analysis——The lyophilized dextranase preparation (about 5 mg) was hydrolyzed in a sealed evacuated tube with 1 ml of 6N HCl at 110° for 24, 30 and 48 hr. The hydrolysates were freed of HCl by drying on a rotary evaporator at 40° and analyzed with a Hitachi Amino acid Analyzer (model KLA-3B). Tryptophan was determined by spectrophotometrical method[11] and colorimetric method using *p*-dimethylaminobenzaldehyde.[12] Sulfhydryl group was estimated by the method of Robyt, *et al.*[13] using 5,5′-dithio-bis(2-nitrobenzoic acid) (DTNB). For the determination of masked sulfhydryl group, the estimation was also carried out in 2% sodium dodesylbenzenesulfonate (SDS) and 6M guanidine hydrochloride. The S-S bond was reduced with 2-mercaptoethanol according to the method of Anfinsen and Harber,[14] and then the sulfhydryl groups were determined as above.

Determination of Sugars and Clarification of Glycoprotein——Neutral sugar content in the enzyme was estimated by phenol–H_2SO_4 method[15] and glucosamine was also determined with the procedure of Elson-Morgan.[16] After polyacrylamide disc electrophoresis by the method of Davis,[17] glycoprotein in the gel was treated with periodic acid followed by staining with fuchsin-sulfite.[18] The glycoprotein was demonstrated as a red-purple band. The protein was also stained with amidoschwarz 10B.

P. funiculosum dextranase



B. fuscum dextranase

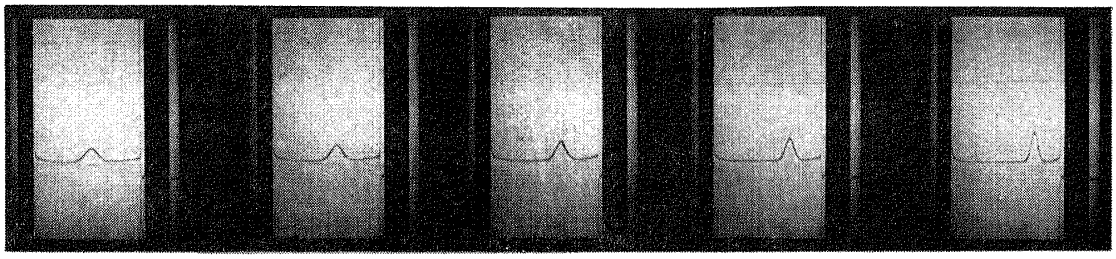


Fig. 1. Ultracentrifuge Run Dextranases from *B. fuscum* and *P. funiculosum*

The photographs were taken from right to left at 10 min intervals after reaching full speed 55430 rpm. The concentrations of dextranases from *B. fuscum* and *P. funiculosum* were 0.47 % and 0.54 % in 100 mM phosphate buffer (pH 7.5) and (pH 6.0) containing 10 mM NaCl, respectively.

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9) O. Vesterberg and H. Svensson, *Acta Chem. Scand.*, **20**, 820 (1966).
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16) G. Blix, *Acta Chem. Scand.*, **2**, 467 (1948).
17) B.J. Davis, *Ann. N.Y. Acad. Sci.*, **121**, abstr., **2**, 404 (1964).
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Results and Discussion

Sedimentation Analysis

The sedimentation patterns of dextranases from *B. fuscum* and *P. funiculosum* are presented in Fig. 1. The purified enzymes showed one peak throughout an analysis at 55430 rpm. The sedimentation constants ($S_{20,w}$) of these dextranases in 100 mM phosphate buffer (pH 7.5 for *B. fuscum* and pH 6.0 for *P. funiculosum*) containing 10 mM NaCl were found to be about 4.35S and 4.37S, respectively.

Isoelectric Point (pI), Molecular Weight and Intrisic Viscosity

In the previous paper,[3] we have reported that dextranase from *P. funiculosum* was fractionated into two preparations, dextranases I and II, by isoelectric focusing and that isoelectric points of the enzymes were found to be 3.98 and 4.19, respectively. On the *B. fuscum* dextranase, isoelectric focusing was also performed for 48 hr with a potential 700 V at 4° and the enzyme was found to be an acidic protein with pI 4.17. Therefore, isoelectric points of these enzymes were very similar to those of dextranases from *Aspergillus carneus* (pI 4.12) and *P. luteum* (pI 4.1) reported by Hiraoka *et al.*[19] Molecular weight of dextranase was determined by gel filtration on Bio Gel P-100. Results are shown in Fig. 2. The molecular weights, 5.5×10^4 and 4.4×10^4, were calculated for *B. fuscum* and *P. funiculosum* (dextranases I and II), respectively. Viscosities of dextranases were also measured in 10 mM

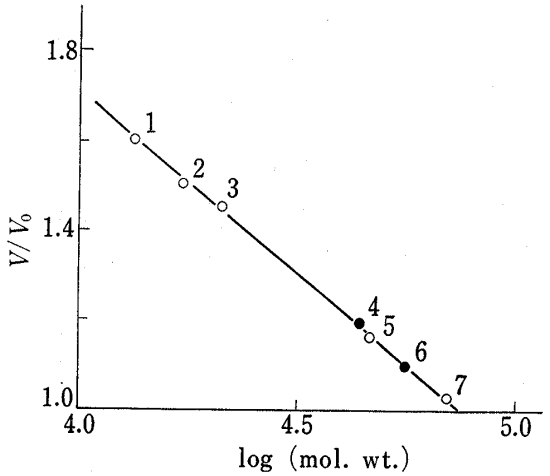

Fig. 2. Determination of Molecular Weight of Dextranases by Gel Filtration on Bio Gel P-100

A column (1.2 cm × 80 cm) of Bio Gel P-100 equilibrated with 25 mM phosphate buffer (pH 7.5) containing 100 mM KCl was used and elution was carried out with the same buffer.
1; cytochrome c (molecular weight 1.3×10^4) 2; myoglobin (molecular weight 1.79×10^4) 3; α-chymotrypsin (molecular weight 2.2×10^4) 4; *P. funiculosum* dextranases I and II 5; egg albumin (molecular weight 4.5×10^4) 6; *B. fuscum* dextranase 7; bovine serum albumin (molecular weight 6.9×10^4)

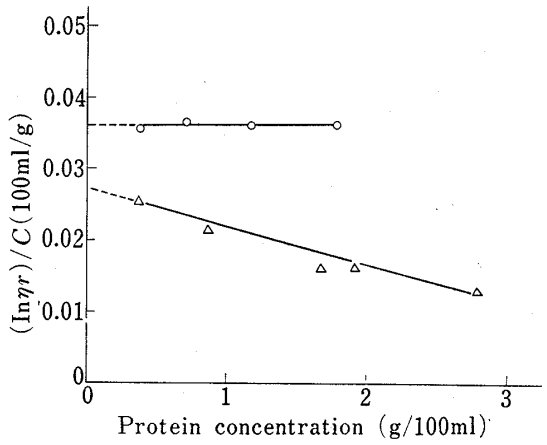

Fig. 3. Plots of the Inherent Viscosities *vs.* Concentrations of Dextranases

Viscosity measurements were carried out in 10 mM phosphate buffer (pH 7.5 for *B. fuscum*) and (pH 6.0 for *P. funiculosum*) containing 100 mM NaCl.
○ :*B. fuscum* dextranase
△ :*P. funiculosum* dextranase

phosphate buffer (pH 7.5 for *B. fuscum*) and (pH 6.0 for *P. funiculosum*) containing 100 mM NaCl. Results are presented in Fig. 3. Inherent viscosity, $(In\gamma\gamma)/C$, was ploted against protein concentration. The inherent viscosity of *B. fuscum* dextranase is independent of the enzyme concentration, and a value of 3.80 ml/g was obtained for the intrinsic viscosity.

19) N. Hiraoka, H. Tsuji, J. Fukumoto, T. Yamamoto and D. Tsuru, *Int. J. Peptide Protein Res.*, **5**, 161 (1973).

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On the other hand, the inherent viscosity of *P. funiculosum* dextranase is dependent of the enzyme concentration, and the intrisic viscosity was found to be 2.70 ml/g.

Ultraviolet Absorption Spectrum

Dextranases from *B. fuscum* and *P. funiculosum* were dissolved in 100 mM phosphate buffer (pH 7.5) and (pH 6.0), respectively, and the absorption spectrum of the solution was recorded on a Hitachi recording spectrophotometer (model 323). As shown in Fig. 4, typical absorption spectra were recorded on both dextranases, respectively. *B. fuscum*; max. 278 nm and min. 252 nm, *P. funiculosum*; max. 280 nm and min. 250 nm. A shoulder was observed at about 290 nm on the *P. funiculosum* spectrum. Extinction coefficient at 280 nm ($E^{1\%}_{1cm}$) were calculated 17.4 for *B. fuscum* and 17.5 for *P. funiculosum*, respectively.

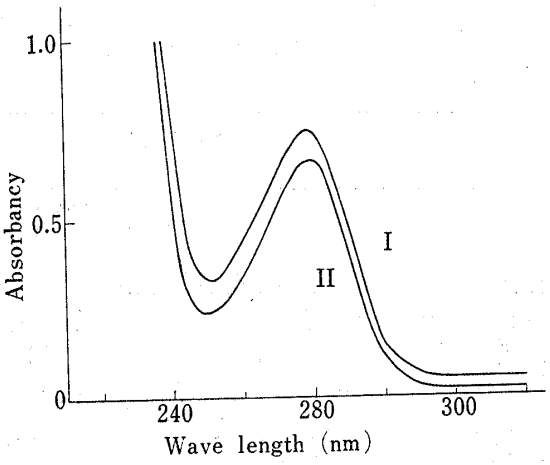

Fig. 4. Ultraviolet Absorption Spectra of Dextranases from *B. fuscum* and *P. funiculosum*

The enzymes from *B. fuscum* (4.2 mg) and *P. funiculosum* (3.8 mg) were dissolved in 10 ml of 100 mM phosphate buffer (pH 7.5 for the former and pH 6.0 for the later) and the absorption spectrum of each solution was recorded on a Hitachi recording spectrophotometer model 323.
I: *B. fuscum* dextranase
II: *P. funiculosum* dextranase

Amino Acid Composition

Lyophilized pure enzyme was hydrolyzed with 6N HCl and performed for amino acid analysis. Results are presented in Table I. When the molecular weight of dextranase

Table I. Amino Acid Composition and Sugar Content of Dextranases from *B. fuscum* and *P. funiculosum*

Amino acid	*B. fuscum* No. of residues per mole (molecular weight 5.5×10^4)	*P. funiculosum* No. of residues per mole (molecular weight 4.4×10^4)
Asp[a)]	54	41
Thr[b)]	33	26
Ser[b)]	34	33
Glu[a)]	42	25
Pro[a)]	22	21
Gly[a)]	32	29
Ala[a)]	25	20
Cys[c)]	0	2
Val[d)]	32	25
Met[d)]	12	10
Ileu[d)]	20	25
Leu[a)]	20	15
Tyr[a,e)]	22	17
Phe[a)]	16	17
Lys[a)]	25	12
His[a)]	12	8
Arg[a)]	12	9
Try[e,f)]	16	11
Sugar[g)]	11	10

a) avarage values from the 24, 30 and 48 hr hydrolysates
b) values extrapolated to zero hydrolysis time
c) colorimetric determination with DTNB
d) Maximum values were adopted.
e) determined spectrophotometrically
f) determined with *p*-dimethylaminobenzaldehyde method
g) expressed as glucose equivalent

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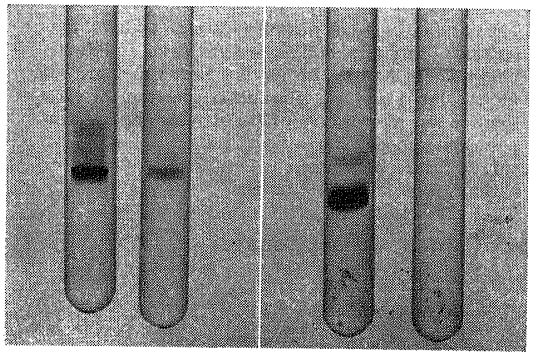
Table II. Determination of Sugar Contents of Dextranases from *B. fuscum* and *P. funiculosum*

Analytical method	*B. fuscum* mole equivalent/mole (molecular weight 5.5×10^4)	*P. funiculosum* mole equivalent/mole (molecular weight 4.4×10^4)
Phenol-H_2SO_4[a]	10.9	10.1
Elson-Morgan[b]	3.1	1.1

a) neutral sugar contents as glucose equivalent
b) amino sugar contents as glucosamine equivalent

from *B. fuscum* and *P. funiculosum* were assumed 5.5×10^4 and 4.4×10^4, respectively, the former enzyme was found to be composed of more than 429 amino acid residues of 17 amino acids and the later contained more than 349 of residues of 18 amino acids. The amino acid compositions were very similar except for cystine. It was a in particular difference that 2 residues of cystine were contained in 1 mole of *P. funiculosum* dextranase, but not in *B. fuscum*. After reduction of *P. funiculosum* dextranase by 2-mercaptoethanol, determination of sulfhydryl groups suggested that a S-S bond and two free sulfhydryl groups were presented in the enzyme.



a b a b
P. funiculosum *B. fuscum*

Fig. 5. Staining Patterns of Dextranases from *B. fuscum* and *P. funiculosum* in Acrylamide Gel

Disc electrophoresis was carried out using pH 9.4 gel. A current of 4 mA/gel was supplied for 70 min at 4°. a: protein stained with amidoschwarz 10B b: glycoprotein staining with periodic acid and fuchsin-sulfite

On the dextranase of *P. funiculosum*, it has been reported that the two free sulfhydryl groups are not essential for the enzyme activity, but the groups are the residue which are concerned activation of the enzyme with Co^{2+}.[6] Sugar contents of the dextranases are presented in Table II. *B. fuscum* dextranase was found to contain 11 moles of neutral sugars as glucose and 3 moles of amino sugars as glucosamine per one mole of the enzyme, and in *P. funiculosum* dextranase, the former was 10 moles and the later was 1 mole. The results of staining of protein and glycoprotein in the disc electrophoresis gels are shown in Fig. 5. On both dextranases, a red-purple band corresponding to each enzyme protein was appeared by the treatment. However, dextranase from *B. fuscum* gave a dimly wide band in spite of using a large amount of protein. From the above observations, both dextranases were suggested to be a glycoprotein as well as the enzymes of *Aspergillus carneus* and *P. luteum*.[19] Chaiet, *et al.* have reported that dextranase from *P. funiculosum* NRRL 1768 was not a glycoprotein,[20] therefore our observation of the enzyme was distinctly different from the description.

To investigate the N-terminal amino acid of the dextranases, dinitrophenylation of the enzymes was carried out in 100 mM $NaHCO_3$ containing 8M urea and N-terminal amino acid was determined by the procedure of Frankel-Conrat.[21] However, we failed to detect the N-terminal amino acid of both dextranases.

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